Angiotensin II promotes human satellite cell proliferation via stimulation of AT2 receptors and TRPC channels

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Regeneration of mammalian skeletal muscle following injury is mediated by satellite cells (SCs) (1). They are normally quiescent in the healthy muscle, but upon post-trauma activation they start to proliferate and fuse with damaged ?bers contributing to tissue repair. A specific microenvironment (cell niche) controls SC quiescence and is involved in their activation during the regeneration process.

There is increasing interest in the potential effects of Angiotensin II (AngII), the main effector of the Renin-Angiotensin System on skeletal muscle. The role of AngII is still debated due to divergent results obtained in previous reports, which propose AngII either as promoter or negative regulator of muscle growth and regeneration (2, 3). Moreover, little information are available on the signaling intermediates, including ion channels, possibly involved in AngII effects on SC. Transient Receptor Potential Canonical (TRPC) channels are emerging as possible candidate being expressed in SC and involved in different biological function such as growth in non-excitable cells (4).

The aim of this study was to assess the role of AngII and cognate receptors (AT1R and AT2R) and the involvement of TRPC channels in the proliferation human SC (hSC).

hSCs were isolated from *pectoralis major muscle* specimens obtained from patients undergoing surgery for *pectus excavatum*. The investigation conforms with the approval by the local ethical committee. Briefly, specimens were digested in 0.2% (wt/vol) collagenase type I/DMEM for 1-2h at 37°C. hSCs were plated in Promocell Growth Medium and splitted when sub-confluent. All the experiments were performed from passage 2 to 5. Cultured hSCs were analyzed by immunofluorescence for the expression of Pax7 and Myf5, transcription factors typical of quiescent/activated SCs. Proliferationand viability were assessed after starvation without serum by cellcounting after hematoxilin-eosin reaction and MTT assay, respectively.

After isolation, hSCs proliferated slowly and reached 80% confluence after 12 days. After the first splitting cells displayed an exponential increase in density and doubled after 24h. A stationary growing phase was reached after 48h. Immunofluorescence analysis indicated that hSCs express both Pax7 and Myf5 in the nucleus. The phenotype did not change over passages indicating that culturing conditions are able to maintain a stable phenotype.

hSCs exposed to AngII (100nM) displayed an enhanced proliferation at 24 and 48h, where number raised to 32% and 61% compared to cells grown in basal medium. Treatment with irbesartan (1μM), an AT1R antagonist, was ineffective to prevent the enhanced proliferation induced by AngII, while PD-123319 (1μM), an AT2R antagonist, completely abolished the effect. MTT assay did not show any significant difference between cells exposed or not to AngII, which evidenced a similar increase of mitochondria activity. Treatment with SKF96365, a pan TRPCinhibitor, abolished the enhanced proliferation of hSCs, suggesting that TRPC are signaling mediator of AngII.

These findings demonstrate that AngII promotes hSCs proliferation via activation of AT2R and TRPC channels stimulation and open new perspectives for individuating new pharmacological tools to improve resident SC homeostasis and self-renewal. Future experiment will clarify the potentiality of AT2R/TRPC channels signaling to improve SC differentiation and regenerative capacity.

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1. Kuang S et al., Trends Mol Med. 2008;14(2):82-91
2. Johnston AP et al., PLoS One.2010;5(12):e15212
3. Yoshida T et al., J Biol Chem 16;288(33):23823-32
4. Shapovalov G et al., Cell Calcium. 2011;50(3):295-302.